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Activity and Local Delivery of Azithromycin in a Mouse Model of *Haemophilus influenzae* Lung Infection

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We compared the activities of azithromycin and erythromycin against *Haemophilus influenzae* in a mouse model of nonparenchymatous lower respiratory tract infection. In vitro and in vivo efficacy data for both drugs were analyzed relative to their pharmacokinetics in lungs and in vivo uptake by phagocytes. Aged C57BL/6 mice (mean age, 15.1 ± 1.9 months) were infected intratracheally with 10^8 CFU of *H. influenzae* serotype b. Oral drug administration was initiated 4 h after infection by various dosage regimens. In terms of bacterial killing in the lung, azithromycin was much more active than erythromycin ($P < 0.01$). Its in vivo activity was also more durable after a single administration relative to the durability of three doses of erythromycin given at 6-h intervals. The MIC of azithromycin was eightfold lower than that of erythromycin, and better penetration and a longer half-life in lung tissue were achieved after a single oral administration. Phagocytes delivered increased amounts of both drugs to the infected lungs, particularly at the site of infection (bronchoalveolar airspaces), and detectable levels of azithromycin were maintained locally for long periods. The fact that the efficacy of azithromycin coincided with the arrival of large numbers of polymorphonuclear leukocytes within the airspaces suggests that active extracellular concentrations were provided by the release of azithromycin from these cells. This further supports the potential value of once-daily azithromycin regimens for the treatment of lower respiratory tract infections in humans, provided that inhibitory concentrations against common pathogens such as *H. influenzae* are maintained for adequate periods of time.

Macrolide antibiotics are commonly used for the treatment of respiratory tract infections because of their broad spectra of activity against both intracellular and extracellular pathogens and their good safety. However, they show poor in vitro activity against *Haemophilus influenzae* (12), and despite advantageous phamacokinetics in the respiratory tract (1), this poor activity has been responsible for clinical failures in patients with otitis, sinusitis, and acute exacerbations of chronic obstructive pulmonary diseases. Azithromycin is a new 15-member-ring macrolide, the first of a novel subclass referred to as the azalides, and shows significantly improved potency against gram-negative bacteria (12, 16). Azithromycin has an unusual intracellular disposition, resulting in improved pharmacokinetics in tissue, with high and sustained levels after oral dosing (7, 8, 17). The aim of this study was to compare the in vivo bactericidal activity of azithromycin against *H. influenzae* with that of erythromycin in a mouse model of pulmonary infection. Experimental models of *H. influenzae* pneumonia are scarce and are generally associated with high-amplitude bacteremia and early mortality (4) that do not correspond to the therapeutic indications for macrolides. We therefore used aged mice and a sublethal inoculum, resulting in nonparenchymatous lower respiratory tract disease. The drugs were evaluated for their abilities to eradicate bacteria from the lungs. The activities of both drugs in vivo and in vitro were analyzed relative to their pharmacokinetics in the lung, and uptake of the drugs by phagocytes in vivo was evaluated in cell pellets obtained by bronchoalveolar lavage (BAL).

(Part of this work was presented at the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy [18].)

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MATERIALS AND METHODS

Animals. Female C57BL/6J mice (age 12 to 17 months; mean ± standard deviation [SD], 15.1 ± 1.9 months) were bred and reared under specific-pathogen-free conditions at Iffa-Credo Laboratories (L'Arbesle, France). They were housed in regulation animal quarters at the Institut National de la Santé et de la Recherche Médicale for 1 week before challenge. In preliminary studies, these mice showed significantly slower pulmonary clearance of *H. influenzae* than did young mice (6 to 7 weeks of age).

Challenge organism. A β-lactamase-producing *H. influenzae* type b strain (87169, which was kindly provided by H. Dabernat, Hôpital Purpan, Toulouse, France) isolated from cerebrospinal fluid was stored at −80°C in 10% skim milk; virulence was enhanced by monthly passage in mice.

Antibiotics. The drugs used in this study were erythromycin (Abbott Lab., North Chicago, Ill.) and azithromycin (Pfizer Laboratories, Groton, Conn.). For in vitro tests, the free-base forms of the drugs were dissolved in methanol and were then diluted in phosphate buffer (pH 8). The drugs were administered orally in water (erythromycin lactobionate) or in a standard diluent (azithromycin base) consisting of methylcellulose (Methocel 15 centipoise) (0.5 g), Tween 80 (1.0 g), low-viscosity carboxymethyl cellulose (10.0 g), sodium chloride (9.0 g), and water (984 ml) (7).

In vitro susceptibility tests. MICs and MBCs were determined by using a broth microdilution method with brain heart infusion supplemented with 2% Fildes enrichment medium (Difco, Detroit, Mich.) and two different inocula (1

$\times 10^6$ and 5×10^7 CFU/ml). The MIC was defined as the lowest antibiotic concentration associated with no visible growth after incubation at 37°C for 24 h. The MBC was determined by plating 0.01 ml from each well onto supplemented chocolate agar plates (BioMerieux, Charbonnières-les-Bains, France) with a Steers replicator and was defined as the lowest antibiotic concentration associated with no colony growth after 24 h.

Time-kill curves were determined by using supplemented brain heart infusion and a starting inoculum of 10^6 CFU/ml. Bacteria were incubated with various drug concentrations (0.5, 1, 2, and 4× the MIC). Bacterial counts were determined by plating 0.1 ml of serial 10-fold dilutions onto chocolate agar at 0, 1, 3, 5, 7, and 24 h. A bactericidal effect was defined as <0.01% survival of the initial inoculum.

Mouse infection model. Animals were infected by intratracheal instillation via the mouth as described elsewhere (4). Briefly, mice were anesthetized intraperitoneally with 0.2 ml of 0.65% sodium pentobarbital and were suspended by the upper incisors. The trachea was cannulated via the mouth with a blunt needle, and 50 μl of bacterial suspension (10^8 CFU per mouse) was instilled. The animals were kept vertical for about 5 min to facilitate distal alveolar migration.

The challenge was made by using an exponential-log-phase, 6-h culture. The bacteria were washed twice and were resuspended in saline at the appropriate density. The size of the inoculum was confirmed by serial dilution and quantitative subculture. The efficacy of inoculation was systematically tested by quantitation of viable organisms in the lung 4 h after infection (the standard error relative to the theorical inoculum was <3%; $n = 3$). Infection was characterized on the basis of bacteriological and histopathological criteria.

Bactericidal activity in vivo. The study drugs were assessed for their ability to eradicate bacteria from the lung. Drug administration was initiated orally, by gavage, 4 h after infection and consisted of a single dose (azithromycin) or two or three doses given once every 12 or 6 h, respectively. Various doses (25, 50, or 100 mg/kg of body weight) of each drug were administered in 0.5 ml of diluent; untreated controls received the same volume of isotonic saline. The total CFU recovered from whole-lung homogenates was determined immediately before the start of treatment (H + 4), 12 h after the first administration (H + 16), 24 h after the end of treatment, and 36 h after the single administration (H + 40) in both treated and untreated animals. Mice were killed by CO_2 asphyxiation and were exsanguinated by cardiac puncture. The lungs were removed and homogenized in 2 ml of saline. Serial 10-fold dilutions of the homogenates were plated onto chocolate agar (0.1 ml per 9-cm-diameter plate). Results are expressed as the mean ± SD of log CFU per lung; each group consisted of three mice. The lower limit of detection was 2 log CFU per lung, which corresponded to the lowest dilution of tissue homogenates (10^{-1}) that avoided significant drug carryover with control inocula.

Pharmacokinetic studies. The pharmacokinetic parameters of the study drugs were examined simultaneously in healthy controls and infected mice. The concentrations of azithromycin and erythromycin in lungs and sera were determined after a single oral dose of 50 mg/kg. In infected mice, the antibiotic was given 4 h after infection. Sera and lungs were collected from mice ($n = 3$) at 0.5, 1, 3, 5, 7, 24, 48, 72, and 96 h following drug administration. The animals were killed by diethyl-ether inhalation and were exsanguinated by cardiac puncture. Blood samples were centrifuged and serum was collected. Lungs were harvested from exsanguinated mice, washed in saline, and then homogenized in 1 ml of phosphate buffer (pH 8). Homogenates were centrifuged, and the supernatants were used for the assay. Drug concentrations were determined by a bioassay with *Micrococcus luteus* ATCC 9341 as the test organism and antibiotic medium 11 (Difco). Standards were made in phosphate buffer in order to evaluate the active unbound fraction of the drug. However, protein binding of azithromycin and erythromycin is low in mouse serum (7.2 and 19%, respectively, at a drug concentration of 0.5 μg/ml) (17) and is saturated at a concentration of 0.5 μg of azithromycin per ml. The proportion of known amounts of drug recovered from tissues was ≥90% for azithromycin and ≥85% for eythromycin, with concentrations ranging from 32 to 0.5 μg/g. The calibration curve was linear from 0.06 to 32 μg/ml. The sensitivity of the bioassay was 0.06 μg/ml for the two drugs. Within- and between-day variations on replicate samples ($n = 5$) were <10%.

Pharmacokinetic analysis. Serum concentration-time data were fitted to one- or two-compartment open models according to the curves of plotted data, and parameters were estimated by standard methods (11). C_{max} is the maximal concentration observed; T_{max} is the time to C_{max}; $t_{1/2\beta}$ is the terminal elimination half-life calculated by using linear least-square regression for the $\log_e$-linear terminal elimination phase; AUC_{0-24} is the area under the curve from zero to 24 h determined by using the trapezoidal rule. The disposition rate of drug from tissue was estimated by using the least-square regression analysis of $\log_e$ concentration against time (half-life = 0.693/slope of the $\log_e$-linear terminal elimination phase).

Uptake of antibiotics by phagocytes in vivo. The intracellular concentrations of the two drugs were evaluated in phagocytes obtained by BAL at 1, 3, 5, 7, 24, 48, 72, and 96 h after administration of a single oral dose (50 mg/kg). In infected mice, the drugs were administered 4 h after infection. The mice were killed by CO_2 asphyxiation and were exsanguinated by cardiac puncture. Lungs were washed six times with 0.5 ml of isotonic saline at 4°C after cannulation of the trachea. The different fractions were pooled, and BAL fluid was centrifuged at 1,700 × *g* for 10 min (4°C). Cell pellets were stored at −80°C until they were assayed. Prior to centrifugation, cells were counted in a portion of BAL fluid by using a hemacytometer (SD, 25 to 30%; $n = 3$); differential cell counts were made by using Giemsa-stained cytocentrifuge preparations. Prior to assay, cell pellets were dried at 37°C, resuspended in 20 μl of buffer (pH 8), and lysed by sonication. Lysates were used for the drug assays described above. The amount of cell-associated antibiotic per cell pellet was determined, and the intracellular concentration was calculated relative to the differential cell number and corresponding cell volume. The assumptions were made that drug was homogeneously distributed within cells and that the amount of intracellular water was equal to the cell volume, i.e., 2.0 and 10.9 μl/10^7 cells for polymorphonuclear neutrophils (PMNs) and alveolar macrophages (AMs), respectively (8). Results were expressed as micrograms per milliliter of intracellular water.

Statistical analysis. Differences in CFU between the treated and untreated groups were evaluated by analysis of variance and the Mann-Whitney U test for nonparametric analysis ($n = 3$). All analyses were conducted with Version II Statview SE software (Abacus Concepts, Inc). *P* values of 0.05 or less with the two tests were considered statistically significant.

TABLE 1. Efficacy of study drugs on pulmonary clearance of *H. influenzae* after oral administration initiated 4 h after infection[a]

Antibiotic	Dose (mg/kg)	Time of dosing (h)	Mean log CFU/lung (mean ± 1 SD)[b]		Mean log reduction in CFU at H + 40
			H + 16	H + 40	
Saline (control)			7.76 ± 0.40	6.93 ± 0.02	0
Erythromycin	25	4, 16	7.91 ± 1.30	5.63 ± 0.60[c]	1.30[c]
	50	4, 16	7.32 ± 1.10	5.39 ± 0.18[c]	1.54[c]
	100	4, 16	6.73 ± 0.79	4.63 ± 0.90[c]	2.30[c]
Azithromycin	25	4, 16	7.88 ± 0.49	4.72 ± 1.04[c]	2.21[c]
	50	4, 16	7.43 ± 0.41	2.00 ± 0.18[c,d]	4.93[c,d]
	100	4, 16	5.86 ± 0.26[c]	<2.00[c,d]	>4.93[c,d]
Erythromycin	50	4, 10, 16	ND[e]	4.25 ± 0.30[c]	2.68[c]
Azithromycin	50	4	7.41 ± 0.59	4.38 ± 0.29[c]	2.55[c]

[a] The lungs of infected control mice contained 8.19 ± 0.09 log CFU at the onset of treatment (n = 5).
[b] Values are means ± SDs (n = 3). H + 16, 12 h after a single administration; H + 40, 24 h after the end of treatment or 36 h after a single administration (azithromycin).
[c] Significantly different from the result for untreated controls ($P < 0.05$).
[d] Significantly different from all other treated groups ($P < 0.01$).
[e] ND, not determined.

RESULTS

In vitro tests. The MICs and MBCs for the *H. influenzae* challenge strain were 2 and 4 μg/ml, respectively, and for erythromycin and 0.25 and 0.25 μg/ml, respectively, for azithromycin. The size of the inoculum (1×10^6 or 5×10^7 CFU/ml) did not influence these values. Time-kill studies showed that azithromycin had the most rapid bactericidal effect, with ≥99.99% killing at 1 μg/ml (4 times its MIC) in less than 7 h. Erythromycin was bactericidal in 18 h at 4 times its MIC (8 μg/ml).

Mouse infection model. Infection resulted in early edema and infiltration of the airways by PMNs. Infiltration was massive at 24 and 48 h, predominantly in the alveolar and peribronchovascular spaces, without thickening of the alveolar walls. Lung weight was increased by 60% at 24 h relative to that in noninfected controls (mean ± SD weight, 0.2606 ± 0.0149 versus 0.1623 ± 0.0178 μg). Bacterial clearance correlated well with the PMN infiltration in the airways, and this could partly explain the significantly slower clearance in aged mice relative to that in young mice (1 $\log_{10}$ CFU reduction relative to the initial bacterial inoculum versus 4 $\log_{10}$ CFU reduction at 48 h). Bacteria appeared to be limited to the bronchoalveolar spaces, with more than 99% of viable organisms recoverable by BAL at all times after infection. In the absence of apparent parenchymal involvement and bloodstream invasion (all animals had negative blood cultures at 24 h), *H. influenzae* was naturally cleared from the airways in 5 days. Physical improvement coincided with the increase in the body weights of the infected mice from 4 days after infection onward (mean ± SD weight loss; 25% ± 5%). The body weights of infected mice reached that of noninfected control mice 10 days after challenge.

In vivo bactericidal activity. The drugs were assessed for their abilities to clear bacteria from the lungs of treated mice in comparison with their abilities to clear bacteria from the lungs of untreated controls (Table 1). Azithromycin given orally 4 h after infection was more effective than erythromycin. Doses of erythromycin in two administrations that were fourfold higher than those of azithromycin were required to obtain the same log CFU reduction. The activity of azithromycin was also more durable, as shown by a 2.55-log-unit reduction in CFU 36 h after a single administration, despite a lack of effect during the first 12 h. Only three doses of erythromycin at 6-h intervals gave the same level of activity (−2.68 log CFU) over the same period.

Pharmacokinetics. Relevant pharmacokinetic data for azithromycin and erythromycin in sera and lungs following the administration of a single 50-mg/kg oral dose to noninfected and infected mice are given in Table 2. In healthy controls, the C_{max} of azithromycin in serum was significantly lower than that of erythromycin (2.2 ± 0.3 versus 4.5 ± 1.3 μg/ml), but azithromycin showed a longer $t_{1/2\beta}$ (6.8 versus 1.0 h), and detectable levels of the drug were present 24 h postdose (0.1 ± 0.1 μg/ml) compared with only 7 h for erythromycin (0.2 ± 0.1 μg/ml). The $t_{1/2\beta}$ of azithromycin was even longer in the lungs (12.7 h), while erythromycin was rapidly eliminated from both the lungs and serum. The T_{max} of azithromycin occurred later than did that of erythromycin in the lungs (3 versus 1 h). Tissue penetration, i.e., the tissue AUC/serum AUC ratio, was much higher for azithromycin (15 versus 3), resulting in sustained levels 48 h postdose (1.0 ± 0.3 μg/g), a time at which drug levels in serum were below the detection limit.

The pharmacokinetic differences described above were accentuated in infected mice. The penetration of erythromycin into tissue was apparently increased, resulting in a greater tissue AUC (45.2 versus 26.9 μg · h/g), but its $t_{1/2\beta}$ remained unchanged in the lungs and serum (1.2 and 1.0 h, respectively). In contrast, the T_{max} of azithromycin in the lungs of infected mice was delayed to 24 h after administration, with sustained levels (>10 μg/g) from 3 to 48 h after administration. The terminal $t_{1/2\beta}$ of azithromycin from the lungs of infected mice was prolonged relative to that in controls (21.5 versus 12.7 h), and levels were still measurable 96 h after administration (1.9 ± 0.1 μg/g); levels in serum were below the detection limit after 48 h. Finally, the AUC of azithromycin in tissue was increased fourfold in comparison with that in tissue of noninfected mice.

In vivo uptake of azithromycin and erythromycin by phagocytes and release at the site of infection. The intracellular dispositions of azithromycin and erythromycin were evalu-

TABLE 2. Pharmacokinetics of azithromycin and erythromycin in lung and serum after a single oral dose of 50 mg/kg[a]

Treatment group and drug	Site of infection	C_{max} (μg/ml or μg/g)[b]	T_{max} (h)	$t_{1/2\beta}$ (h)	AUC (μg · h/ml or μg · h/g)
Noninfected control mice					
Azithromycin	Serum	2.2 ± 0.3[b]	1	6.8	13.6
	Lung	13.6 ± 2.7	3	12.7	201.2
Erythromycin	Serum	4.5 ± 1.3	0.5	1.0	8.0
	Lung	11.2 ± 5.3	1	1.2	26.9
Infected mice[c]					
Azithromycin	Serum	4.1 ± 0.4	1	14.8	31.8
	Lung	12.6 ± 3.5	24	21.5	811.2
Erythromycin	Serum	5.5 ± 1.9	0.5	1.0	10.7
	Lung	15.6 ± 7.7	0.5	1.2	40.7

[a] C_{max}, peak concentration of drug; T_{max}, time to peak concentration; $t_{1/2\beta}$, estimated terminal elimination half-life; AUC, area under the curve.
[b] Values are means ± SDs for three mice.
[c] Drug was administered 4 h after infection.

ated in BAL cell pellets after oral treatment with 50 mg of each drug per kg (Tables 3 and 4, respectively). The pharmacokinetics of the two drugs in AMs were evaluated in noninfected mice (all BAL cells were AMs). Estimated intracellular concentrations revealed a marked macrophage uptake of erythromycin (five to eight times the concentrations in the whole-lung homogenates) but a rapid release indicated by the T_{max} and $t_{1/2\beta}$, which were similar to those for serum (T_{max} = 1.0 h; $t_{1/2\beta}$ = 1.5 and 1.0 h in AMs and serum, respectively). In contrast, azithromycin was still concentrated in AMs 24 h after administration (C_{max} = 122.6 μg/ml, which was 47 times the corresponding level in pulmonary tissue), at a time when concentrations in lung had already decreased and levels in serum were near the detection limit. Azithromycin was released very slowly from macrophages, with a $t_{1/2\beta}$ of 23.9 h in these cells compared with 6.8 h in serum and 12.7 h in the lungs.

In infected mice, the amount of cell-associated erythromycin was increased approximately twofold relative to that in healthy controls, but it fell with a decrease in levels in serum (erythromycin was undetectable 7 h after administration). In contrast, the total amount of cell-associated azithromycin increased from 1 to 24 h after drug administration as PMNs migrated into the bronchoalveolar spaces. By 24 h postdose, it was increased fourfold relative to that in noninfected controls. The concentrations of azithromycin in whole-lung homogenates over time appeared to be closely related to cell-associated drug levels in BAL pellets. The intracellular levels of azithromycin were decreased in comparison with those in noninfected controls, possibly because of lower uptake by PMNs than by AMs or because of enhanced release during phagocytosis (8).

DISCUSSION

The use of an experimental model of lung infection to evaluate drug efficacy in terms of pulmonary clearance confirmed the higher in vitro activity of azithromycin against *H. influenzae* relative to that of erythromycin (10, 12, 16). Given orally twice daily, azithromycin (25 mg/kg) was as active as erythromycin given at fourfold higher doses (100 mg/kg). At higher doses, azithromycin eradicated bacteria from the lung more rapidly than did erythromycin. These results were similar to those obtained in a middle-ear infection model in gerbils, in which the 50% effective dose ratio (doses that reduced bacterial counts by ≥99.5% in the ears of 50% of the animals tested) between the two compounds was greater than 3 (36.7 and >100 mg/kg for azithromycin

TABLE 3. Effect of intracellular uptake and phagocyte infiltration evaluated in BAL fluid on pulmonary concentrations of azithromycin

Time (h) after drug administration[a]	Noninfected controls				Infected mice			
	BAL cell pellets[b]			Concn (μg/g) in lung[b]	BAL cell pellets			Concn (μg/g) in lung
	No. of PMNs (10^5)	No. of AMs (10^5)	Intracellular azithromycin (10^{-3} μg)[c]		No. of PMNs (10^5)	No. of AMs (10^5)	Intracellular azithromycin (10^{-3} μg)	
1	0	1.1	3.7 (33.7)	11.1	14.1	0.5	11.6 (37.8)	8.2
7	0	1.7	18.4 (102.5)	6.0	44.6	0.3	37.4 (40.7)	12.0
24	0	1.8	23.3 (122.6)	2.6	71.5	1.5	85.9 (54.0)	12.6
48	0	1.8	14.0 (73.5)	1.0	57.0	1.2	39.5 (31.1)	10.3
72	0	1.8	5.8 (30.4)	0	46.4	3.5	36.9 (28.4)	5.7
96	0	1.8	3.1 (16.1)	0	13.8	3.2	14.9 (24.0)	1.9

[a] Drug was administered orally as a single dose of 50 mg/kg. Infected mice were treated 4 h after infection.
[b] Values are means (n = 3; SD, 25 to 30%).
[c] Total amount of azithromycin in cell pellets. Values in parentheses are the estimated intracellular concentration in phagocytic cells, expressed as micrograms per milliliter of intracellular water.

TABLE 4. Effect of intracellular uptake and phagocyte infiltration evaluated in BAL fluid on pulmonary concentrations of erythromycin

Time (h) after drug administration[a]	Noninfected controls				Infected mice			
	BAL cell pellets[b]			Concn (μg/g) in lung[b]	BAL cell pellets			Concn (μg/g) in lung
	No. of PMNs (10^5)	No. of AMs (10^5)	Intracellular erythromycin (10^{-3} μg)[c]		No. of PMNs (10^5)	No. of AMs (10^5)	Intracellular erythromycin (10^{-3} μg)	
1	0	1.4	7.6 (55.5)	11.2	16.0	0.7	18.0 (50.0)	15.6
3	0	1.7	3.8 (15.0)	2.9	26.0	0.5	10.3 (18.5)	4.4
5	0	1.9	2.1 (9.6)	1.2	32.0	0.5	4.2 (9.8)	1.6
7	0	1.8	ND[d]	ND	27.0	0.6	1.1 (4.8)	0.5
24	0	1.8	ND	ND	88.7	4.2	ND	ND

[a] Drug was administered orally as a single dose of 50 mg/kg. Infected mice treated 4 h after infection.
[b] Values are means (n = 3; SD, 25 to 30%).
[c] Total amount of erythromycin in cell pellets. Values in parentheses are the estimated intracellular concentration in phagocytic cells, expressed as micrograms per milliliter of intracellular water.
[d] ND, below the limit of detection.

and erythromycin, respectively) (7). Our data confirm the pharmacokinetic advantages of azithromycin previously reported in humans (5) and animals (7, 17). In healthy mice, its penetration into tissue was 5-fold greater and its $t_{1/2\beta}$ from tissue was 10-fold longer relative to the values for erythromycin. These differences were accentuated in the infected lung. The pharmacokinetic parameters of erythromycin were not greatly modified by infection, while the penetration into tissue and sequestration of azithromycin were enhanced; stable levels of azithromycin were observed from 3 to 48 h postdose (≥10 μg/g) and its terminal $t_{1/2\beta}$ was twice that in controls.

With regard to the in vitro data and the pharmacokinetics of azithromycin in the lung, it is interesting that its ability to eradicate *H. influenzae* from the lung was not evident 12 h after a single administration, possibly because of the intracellular disposition of azithromycin and the extracellular location of *H. influenzae* (within the airspaces). Indeed, when treatment was started early after infection, the activity of azithromycin closely paralleled PMN infiltration and the amounts of cell-associated drug in the bronchoalveolar spaces. A similar observation was made in the gerbil model mentioned above (6), in which little or no activity occurred in the absence of local inflammation. The intracellular pharmacokinetics of azithromycin in our study were in keeping with in vitro data (8), as follows. (i) Azithromycin was more strongly concentrated by cells than was erythromycin. (ii) Azithromycin uptake continued for at least 24 h. In contrast, the uptake of erythromycin by AMs and PMNs was virtually complete within 1 h. It has also been shown in vitro that erythromycin and azithromycin concentrate not only in phagocytic cells but also in fibroblasts and human lung cells (9, 14). Azithromycin is released very slowly from fibroblasts relative to the release rate of erythromycin, and it is tempting to speculate that these cells may serve as a reservoir of drug that can be used by migrating phagocytes (9). (iii) The release of azithromycin from PMNs and AMs continued at a time when the drug was undetectable in serum, whereas erythromycin rapidly egressed from the cells (in parallel with its elimination from the lung and serum). These results confirm the targeted and prolonged delivery of azithromycin by phagocytes. The time lag in the activity of an early dose of azithromycin until large amounts of cell-associated drug gained access to the airspaces strongly points to its release from phagocytes at inhibitory concentrations. Indeed, in vitro studies have shown that intracellular uptake does not inactivate azithromycin and that the intracellular killing activity of AMs against opsonized pathogens such as *Staphylococcus aureus* is similar whether or not they are loaded with azithromycin (8). The activity of azithromycin was thus more dependent on maintained local extracellular levels, which may act in conjunction with normal phagocytic bacterial clearance mechanisms. Such levels could be favored by the more rapid release of azithromycin by phagocytes in the presence of bacteria, as has been shown in vitro (8). A similar phenomenon may have occurred in this study, since intracellular levels of drug in infected mice were lower than those in noninfected controls.

In conclusion, azithromycin appears to be the most effective macrolide against *H. influenzae*; the antimicrobial activities, intracellular concentrations, and half-lives of macrolide antibiotics may thus determine their in vivo efficacies not only against intracellular pathogens but also against extracellular pathogens. Roxythromycin, for example, reaches high intracellular levels, but it is released as rapidly as erythromycin is (2) and is poorly active in vitro against *H. influenzae* (12); spiramycin is slowly released from cells (13) and has advantageous intracellular pharmacokinetics in vivo (15), but its MICs and MBCs for *H. influenzae* are far higher than those of erythromycin. This emphasizes the potential usefulness of azithromycin in respiratory tract infections in which single daily dosage and short treatment may be possible, given its prolonged delivery at sites of infection and potential risk of accumulation (17). However, if effective inhibitory extracellular concentrations fail to be maintained, therapeutic failures may occur with increases in the MIC, as reported in the treatment of acute exacerbations of chronic bronchitis (3). Well-designed trials are now required to determine the relevance of our experimental findings to the clinical setting.

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